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Diurnal Acid Metabolism in the Submerged Aquatic Plant, *Isoetes tuberculata*

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Diurnal acid metabolism has been studied largely in xerically adapted, terrestrial, succulent plants that carry out Crassulacean Acid Metabolism (CAM) (Kluge and Ting, 1978). In the CAM process, CO_2 is fixed into malic acid during the dark and the activity of the enzyme PEP carboxylase is enhanced. During daylight hours, decarboxylation of CO_2 fixed in the dark takes place, and it enters into the C_3 cycle.

Keeley (1981) reported for the first time the diurnal acid metabolism in the aquatic plant, *Isoetes howellii* Engelm. Since then this physiological process has been noted in 15 species of *Isoetes* out of the 60 known (Keeley, 1983, 1985, 1987; Keeley and Bowes, 1982; Keeley et al., 1983). In India, the genus *Isoetes* is represented by several species (Gena and Bhardwaja, 1984; Panigrahi, 1981; Pant and Srivastava, 1962; Sharma et al., 1985), and they occur as terrestrial, amphibious, or submerged aquatic plants. Diurnal acid metabolism has been studied for the first time in a submerged aquatic species, *I. tuberculata* Gena and Bhardwaja from the Indian subcontinent, using a simplified method of monitoring changes in the pH of leaf contents during a 48 hour period.

METHODS AND MATERIALS

Well-established, submerged plants of *I. tuberculata* growing in an artificial pond were selected. 50 mg leaf samples were washed with distilled water, dried by pressing on blotting paper, and crushed in distilled water. The pH of the resulting solution was measured with a Toshniwal pH meter. For each plant, this procedure was repeated at 3 hour intervals up to 40 hours, with the utensils carefully washed with distilled water between replicates. The pH of the water in the artificial pond also was measured at the same intervals.

RESULTS AND DISCUSSION

The changes in the pH of leaf contents during different hours of two days and nights are shown in Fig. 1. The pH decreases during the night and increases during the day. This indicates that there is an increase in acidification during the dark, probably due to an accumulation of malic acid. In CAM plants, it is malic acid that increases during the night. This malic acid is utilized during the day, and the pH thus increases then.

During the same period of time, the pH of the pond water also showed slight

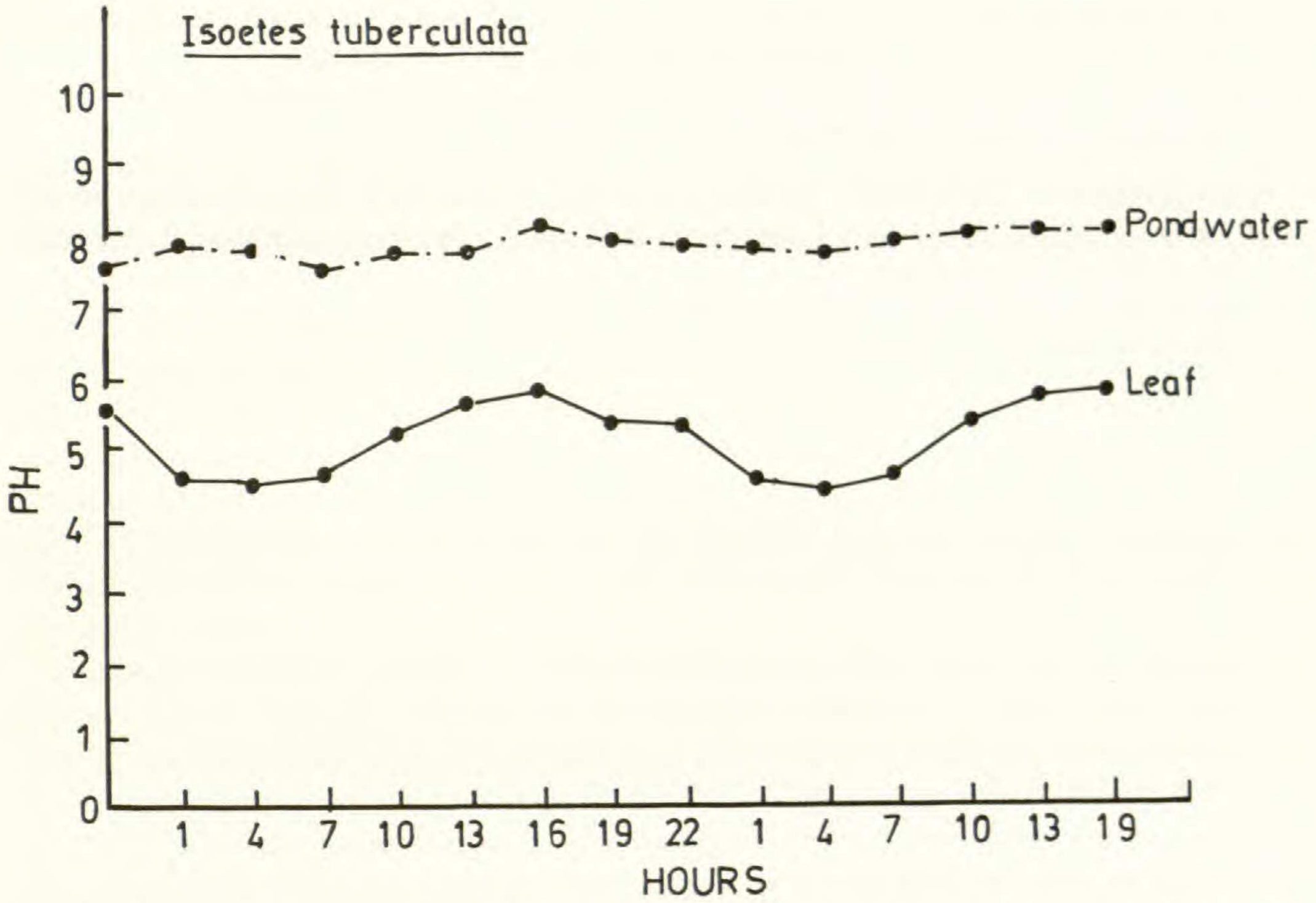

FIG. 1. Changes of pH in the leaves of *Isoetes tuberculata* during the day and night. During the same period, water from the surrounding pond habitat shows negligible changes.

changes during the day and night. However, this variation is negligible (Fig. 1).

Preliminary investigations show that in *I. tuberculata* significant amounts of diurnal acid metabolism occur, and suggest that this species is a CAM plant. However, simple measurement of pH is not a conclusive method for determining the operation of CAM in a species. An absence of pH changes may reflect a well-buffered cytosol, rather than the absence of CAM. Titration (Keeley, 1983) is a more accurate method for elucidating CAM. Further investigations in this regard are being undertaken in this laboratory.

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